11 ULTRAVIOLET SPECTROSCOPY

11.1 Introduction:

11.1.1 Ultraviolet spectroscopy (UV) is a good preliminary screening method for identifying an organic compound with aromatic rings or conjugated systems. However, this method has limited specificity because structurally related compounds give similar spectra.

11.1.2 Light energy absorbed in the ultraviolet region causes the electrons to undergo transitions from ground states to higher-energy states (electronic transitions). These transitions and the corresponding wavelengths of absorbed energy are characteristic of a group of atoms called a chromophore. Addition of substituents with electron withdrawing or donating properties to the conjugated system of the chromophore causes changes in the resulting spectrum.

11.1.3 In UV spectroscopy, the sample is placed between an energy source (Deuterium lamp) and a spectrometer. The source provides electromagnetic radiation in the ultraviolet region (180 - 400 nm). Many UV spectrophotometers are double beam instruments, in which the spectrometer measures the absorbed energy relative to a reference. The reference beam serves to subtract solvent absorptions from the resulting spectrum. Other instruments which contain diode array detectors (e.g., scanning LC detectors) are also allowable.

11.1.4 The choice of solvent is important. The solvent should not absorb ultraviolet radiation in the same region as the sample. Solvents without conjugation such as water (range of pH), 95% ethanol, and n-hexane are most commonly used.

11.1.4.1 These solvents vary as to the shortest wavelength at which they remain transparent to UV radiation.

11.1.4.2 The effects of a solvent on the fine structure of an absorption band should be considered. For example, in a polar solvent the hydrogen bonding forms a solute-solvent complex and the fine structure may disappear.

11.1.4.3 Sometimes the spectrum in an acidic medium is different from the spectrum in a basic medium. The change in absorption maxima due to a change in pH can be indicative of certain compounds. Examples of compounds which exhibit a distinctive acid-base shift are acetaminophen, morphine, and most barbiturates.

11.1.5 UV spectroscopy can be very useful as a quantitation technique as it is based on the Beer-Lambert Law.

11.2 Sample Preparation:

11.2.1 For drug screening, dissolve the sample in both acidic and basic media. For most drug analyses, suitable solvents are 0.2N H_2SO_4, 0.1N HCl and 0.1N NaOH.

11.2.2 Samples should be relatively pure for UV quantitation. Samples containing more than one absorbing compound may have additive absorbance values. Extraction is the most common method for purification. Samples containing other substances that do not absorb in the UV region of interest need not be removed prior to quantitation.

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